

### **REMARKS**

The Office Action of February 15, 2011, has been carefully studied. Claims 11, 2 and 4-34 currently appear in this application. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicant respectfully requests favorable reconsideration and formal allowance of the claims.

### **Claim Amendments**

Claim 1 has been amended to recite a modified plant including at least one copy of at least **two different** sequences using **two different eukaryotic** P<sub>1B</sub>-type ATPase of the Zn<sup>2+</sup>Co<sup>2+</sup>/Cd<sup>2+</sup>Pb<sup>2+</sup> subclass and that overexpresses the **two different** P<sub>1B</sub>-type ATPase. Support for this amendment can be found in the specification as filed at original claim 3 and paragraph [0030] of the published application.

Claim 3 has been cancelled.

Claims 1, 2, 4, 7 and 8 have been amended in accordance with the Examiner's helpful suggestion to include an article before "genetically..."

Claim 4 has been further amended by replacing the term "encoding P<sub>1B</sub>-type ATPase of the Zn<sup>2+</sup>Co<sup>2+</sup>/Cd<sup>2+</sup>Pb<sup>2+</sup> subclass and at least another sequence" and adding the term, "further", and by deleting the limitations within the bracket and "such as."

Claims 5 and 6 have been amended in accordance with the Examiner's helpful suggestion to recite an article before "recombinant."

Claim 6 has been amended to delete the term "such as."



Claims 1, 4, 5 and 9 have been amended by replacing the term “more than one” by the term “one or more than one.” Support for this amendment can be found in the specification as filed at paragraphs [0029], [0036], [0041] and [0045].

New claim 29 is supported by the specification at paragraph [0030].

New claims 33 and 34 are supported by the specification at paragraph [0037].

### **Specification**

The specification is objected to because it contains embedded hyperlinks.

The present amendment deletes these embedded hyperlinks.

### **Sequence Listings**

A new sequence listing that contains the sequence for Figure 1 is submitted herewith. The description of the Drawings has been amended to include the sequence identifiers.

Attached hereto is a substitute sequence listing in the form of a .txt file. It is requested that this substitute sequence listing be used as both the paper copy and the computer-readable form. Applicants have amended the specification to identify the sequences therein with SEQ ID NOs, and the attached substitute sequence listing includes these sequences, as supported by the specification as filed.

The following statement is provided to meet the requirements of 37 C.F.R. §1.825(a) and 1.825(b).

I hereby state, in accordance with 37 C.F.R. §1.825(a), that the amendments included in the substitute sheets of the sequence listing are believed to be supported in



the application as filed and that the substitute sheets of the sequence listing are not believed to include new matter.

I hereby further state, in accordance with 37 C.F.R. §1.825(b), that the .txt file submitted herewith constitutes both the computer readable form as well as the paper copy of the sequence listing, and therefore they are the same.

Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of



two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Applicants submit that the present application contains patentable subject matter and therefore urge the examiner to pass the case to issuance.

#### **Claim Objections**

Claims 1- 8 and 17 are objected to for lack of articles.

The present amendment includes articles where necessary.

#### **Rejections under 35 U.S.C. 112**

Claims 6 and 13 are rejected under 35 U.S.C. 112, second paragraph, for being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

This rejection is respectfully traversed.

Claim 6 has been amended to delete "such as."

Claim 13 has been amended to delete the narrower range.

Claim 4 has been amended to delete the bracket.

#### **Allowable Subject Matter**

It is noted with appreciation that claim 4 is free of the prior art.



### **Art Rejections**

Claims 1, 3, 5-9, 17 and 20 are rejected under 35 U.S.C. 102(b) as being anticipated by Borremans et al., EP 1 136 558.

This rejection is respectfully traversed.

While Borremans discloses transgenic plants and plant cells comprising nucleotide sequences encoding at least one heterologous heavy metal transport and/or sequestration protein of prokaryotic or eukaryotic origin, as well as nucleotide sequences encoding those proteins, Borremans only discloses examples of P-type ATP genes, as shown in Table 1, page 4:

CadA, CopF, PbrA and SilP genes are of **prokaryotic** origin. Specifically:

- *CadA* is from *Streptococcus aureus* (see Nucifora *et al.* 1989 and Rensing *et al.* 1998 cited in said Table 1),
- *ZntA* is from *Escherichia coli* (see Rensing *et al.* 1997 and 1998 cited in said Table 1),
- *CopF* and *PbrA* are from *Ralstonia metallidurans* (see enclosed article of Borremans *et al.*, J Bacteriol. 2001;183:5651-8).
- *SilP* is from *Salmonella typhimurium* (see Gupta *et al.*, 1999 cited in said Table 1).

The genes identified as Q04656 and U08344 are of **eukaryotic** origin, respectively, from *Homo sapiens* and *Ratus Norvegicus*. **These genes are probably P<sub>1B</sub> type ATPases of the Cu<sup>2+</sup> subclass, but not of Zn<sup>2+</sup>/Co<sup>2+</sup>/Cd<sup>2+</sup>/Pb<sup>2+</sup> + subclass** Indeed, P<sub>1B</sub> ATPases include the two subclasses of the Cu<sup>2+</sup> subclass and the Cd<sup>2+</sup> subclass (see the website <http://www.tranplabs.dk/ptabase/> and the article submitted herewith, Axelsen et al., *J. Mol. Evol.*, 1998, **46**: 84-101).



From the above, it is clear that Borremans does not teach eukaryotic P<sub>1B</sub> type ATPases of the Zn<sup>2+</sup>Co<sup>2+</sup>/Cd<sup>2+</sup>Pb<sup>2+</sup> subclass. Furthermore, Borrenans does not teach a genetically modified plant or a recombinant vector including one or more than one copy of at least two different sequences encoding **two different** eukaryotic P<sub>1B</sub> type ATPases of the Z Zn<sup>2+</sup>Co<sup>2+</sup>/Cd<sup>2+</sup>Pb<sup>2+</sup> subclass.

Reconsideration and withdrawal of the rejection are respectfully requested.

Claims 1-3 and 4-28 re rejected under 35 U.S.C. 103(a) as being unpatentable over Borremans in view of Bernard et al., WO 2004078905 and Chaney et al., US 5,944,872.

This rejection is respectfully traversed.

Claim 1 amended is directed to a genetically modified plant overexpressing at least **two different eukaryotic** P<sub>1B</sub> type ATPases of the Zn<sup>2+</sup>Co<sup>2+</sup>/Cd<sup>2+</sup>Pb<sup>2+</sup> subclass. These genetically modified plants are able to accumulate heavy metals and translocate them in the shoots, and are useful for phytoremediation, particularly for phytoextraction of Zn, Co, Cd or Pb from, a contaminated environment.

Additionally, as shown in Example 3 of the instant application reporting the co-expression of AtHMA3 and AtHMA4 in genetically modified *A. thalian* plants, **these two different eukaryotic P<sub>1B</sub> type ATPases act synergistically.** As a result, the genetically modified plants claimed herein tolerate toxic concentrations of heavy metals over a longer period, hence, enabling a stronger extraction of these metals from contaminated soils.

The technical problem underlying the presently claimed plants is to provide genetically modified plants able to accumulate heavy metals and methods of



removing and possibly recovering these heavy metals, using the genetically modified plants as claimed herein.

As noted above, Borremans discloses genetically modified plants expressing at least one heterogonous P-type ATPase, particularly P-type ATPases of prokaryotic origin and P<sub>1B</sub> type ATPases only of the Cu<sup>2+</sup> classes of eukaryotic origin.

Bernard discloses a list of cDNAs from *Thlaspi caerulescens* encoding proteins involved in cadmium clearance (see pages 14-15 of Bernard). This list includes phytochelation synthase 1, nmetalloprotein type 1, 2 and 3, metalloprotein related protein, P-type ATPase or the Cd<sup>2+</sup>/Zn<sup>2+</sup> subclass, heat shock transcription factor, transcription factor IID, salicylic acid carboxyl methyl transferase, chlorophyll a/b binding proteins, 40S ribosomal protein and Photosystem I subunit.

Bernard also discloses a method for (i) producing transgenic plants (*e.g.*, *A. thaliana* or tobacco plants) expressing a maximum of 4 genes of *T. caerulescens* related to cadmium tolerance and (ii) testing the plants for their phytoextraction capacities. However, Bernard neither discloses nor suggests specific combinations of these genes or the combination of **two different P-type ATPases**. Further, Bernard does not provide any result of using these transgenic plants.

Chaney merely discloses a method for phytoextracting nickel and cobalt from soil by cultivating *Alyssum* plants in the soil.

It is clear from the above that none of the cited patents, alone or in combination, suggests genetically modified plants synergistically overexpressing **two different eukaryotic** P<sub>1B</sub> type ATPases of the Zn<sup>2+</sup>/Co<sup>2+</sup>/Cd<sup>2+</sup>/Pb<sup>2+</sup> subclass.



Additionally, there are no experimental data in the cited patents showing that genetically modified plants overexpressing a P<sub>1B</sub>-type ATPase of the Zn<sup>2+</sup>Co<sup>2+</sup>/Cd<sup>2+</sup>Pb<sup>2+</sup> + subclass would be useful for soil remediation.

It should be noted that WO 02/081707, cited in the present application, at paragraph [0021] discloses genetically modified plants transformed with a recombinant vector comprising a sequence encoding a prokaryotic metal transporting P-type ATPase, in particular, *i.e.*, *E. coli* ZnA protein (which is also disclosed in Borremans). The genetically modified plants disclosed in the '707 application exhibited an increased resistance to heavy metals (*i.e.*, lead and cadmium), but a **decreased uptake of heavy metals** compared to the wild-type plant. Therefore, these genetically unmodified plants cannot be used for phytoremediation, since they do not accumulate heavy metals. As specified in the present application at paragraph [0041], *ZnA* (as well as homologous genes thereof) are not appropriate ATPases for use in phytoremediation.

Hence, based upon the teaching of the '707 application, and on the fact that none of the cited documents provides experimental results showing the effects of the genetic modification, one skilled in the art would have no reason to use to different P<sub>1B</sub>-type ATPases of the Zn<sup>2+</sup>Co<sup>2+</sup>/Cd<sup>2+</sup>Pb<sup>2+</sup> + Pb<sup>2+</sup> subclass to produce genetically modified plants for phytoremediation.

Therefore, one skilled in the art would not have a reasonable expectation of success in using a genetically modified plant overexpressing two different eukaryotic P<sub>1B</sub>-type ATPases of the Zn<sup>2+</sup>Co<sup>2+</sup>/Cd<sup>2+</sup>Pb<sup>2+</sup> subclass for phytoremediation, and that the two different eukaryotic P<sub>1B</sub>-type ATPases of the Zn<sup>2+</sup>Co<sup>2+</sup>/Cd<sup>2+</sup>Pb<sup>2+</sup> subclass would act synergistically. That is, **two different** eukaryotic P<sub>1B</sub>-type ATPases of the Z



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$n^{2+}Co^{2+}/Cd^{2+}Pb^{2+}$  subclass act synergistically and produce superior soil remediation as compared with the cited references.

It is respectfully submitted that the cited references do not contain any information suggesting to one skilled in the art to produce a genetically modified plant overexpressing at least two different eukaryotic  $P_{1B}$ -type ATPases of the  $Zn^{2+}Co^{2+}/Cd^{2+}Pb^{2+}$  subclass. In view of the cited documents, one skilled in the art would have not be motivated to produce such a plant,

It is respectfully submitted that the genetically modified plants as claimed herein are novel and unobvious. The herein claimed method of producing the genetically modified plants, the recombinant vector for transformation said genetically modified plant, and the method of phytoremediation using these genetically modified plants are also believed to be novel and unobvious.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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